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Biopharmaceutics Classification by High Throughput Solubility Assay and PAMPA

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ABSTRACT

The purpose of the present study was to examine the relevancy of the high throughput solubility assay and permeability assay to the biopharmaceutics classification system (BCS). Solubility and permeability were measured by high throughput solubility assay (HTSA) and parallel artificial membrane permeation assay (PAMPA), respectively. High throughput solubility assay was performed using simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid without bile acid (SIF, pH 6.8). We categorize 18 drugs based on the BCS using HTSA and PAMPA. Fourteen out of 18 drugs were correctly classified (78% success rate). The result of the present study showed that HTSA could predict BCS class with a high success rate, and PAMPA could also be useful to predict the permeation of drugs.

Key Words: Solubility; Permeability; High throughput; Biopharmaceutics classification system (BCS).

INTRODUCTION

Solubility and permeability are regarded as important factors for oral drug absorption. Recently, the drug discovery process has progressed with several technologies (e.g., combinatorial chemistry and high throughput in vitro pharmacology screening). Consequently, drug candidates tend to possess large molecular weights, high hydrophobicity, and many hydrogen bonds.[1] Such physicochemical properties often cause low solubility and low permeability that result in poor oral absorption and low bioavailability. Therefore, it is necessary to evaluate solubility and permeability at the early drug discovery stage.

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Table 1. Solubility (S), dose in mg, dose number (Do), and permeability (P_{am} and $P_{PAMPA\text{-}PP\text{-}RF}$) of drug compounds.

No.	Compound name	S_{SGF}[a] (μg/mL)	S_{SIF}[a] (μg/mL)	Dose[b] (mg)	Do[c]	P_{am}[d] ($\times 10^{-6}$ cm/sec)	$P_{PAMPA\text{-}PP\text{-}RF}$[e] ($\times 10^{-6}$ cm/sec)
1	Metoprolol	>653±43	>658±9	150	<0.9	5.67	7.35
2	Propranolol	500±11	489±1	80	0.7	13.7[f]	15.6[f]
3	Dilthiazem	673±6	661±10	120	0.7	39.3[f]	39.7[f]
4	Piroxicam	130±18	>165±0	20	0.6	59.3	59.4
5	Diclofenac	2±0	>330±5	50	111.1	53.3	53.4
6	Verapamil	707±4	695±10	160	0.9	38.4[f]	38.4[f]
7	Ketoprofen	191±7	>471±13	100	2.1	33.8[f]	33.9[f]
8	Naproxen	30±0	>2508±26	500	65.8	49.5[f]	49.7[f]
9	Carbamazepine	268±11	264±7	200	3.0	53.0	54.0
10	Ketoconazole	>261±5	7±1	400	225.4	4.75[g]	4.83[g]
11	Danazol	<1.1	<1.1	400	>1454.5	15.3[f,g]	15.6[f,g]
12	Famotidine	>320±12	>310±6	40	<0.5	<0.05[h]	<1.65[h]
13	Atenolol	>511±17	>499±8	100	<0.8	0.56	2.44
14	Hydrochlorothiazide	>540±21	>599±8	100	<0.7	1.71	2.83
15	Ranitidine	>1576±61	1439±29	300	0.8	1.63	3.01
16	Acyclovir	941±30	>1029±13	400	1.7	<0.04[h]	<1.57[h]
17	Furosemide	19±0	>633±13	80	16.9	3.60	3.71
18	Chlorothiazide	477±18	946±17	500	4.2	0.31	1.34

[a]Values are represented as the mean±S.D. (n=3). The symbol > means that the solubility is larger than 90% of the upper detection limit and the symbol < means that the solubility is less than the lower detection limit. The upper detection limit depends on the sample concentration.
[b]Dose is quoted from dose strength for major use described in the dosage section in American Hospital Formulary Service (AHFS) Drug Information. (From Ref. [12].)
[c]Do was calculated using Eq. (1). The lower solubility between SGF and SIF was used to calculate Do.
[d]Artificial membrane permeability coefficient.
[e]The paracellular pathway corrected P_{am} based on the Renkin function.
[f]The incubation time was 2 hours.
[g]Sodium phosphate buffer contains 30% DMSO.
[h]Less than the detection limit.

Several high throughput screening methods for solubility and permeability were previously reported as in vitro assays. For permeability screening, Caco-2 and Madin-Darby Canine Kidney (MDCK) assays have been widely used as cell-based permeation assays for over 10 years.[2–4] Recently, the parallel artificial membrane permeation assay (PAMPA) has been introduced as a more rapid in vitro assay.[5] We reported a biomimetic variation of PAMPA.[6] Several high throughput solubility assays (HTSA) were introduced in the early drug discovery stage.[7–9] In most cases, phosphate buffered saline (PBS) or pure water has been used as the dissolution media. Compounds were added as the dimethyl sulfoxide (DMSO) solution. Solubility was obtained by means of ultraviolet (UV) spectrometry, high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS) etc., after filtration, or determined in situ by turbidimetry or nephelometry.

It remains unclear how to use the high throughput solubility assay and permeability assay for evaluation of the oral absorption at the early drug discovery stage. The purpose of the present study was to examine the relevancy of high throughput solubility assay and permeability assay to the oral absorption from the viewpoint of the biopharmaceutics classification system (BCS). We tried to categorize 18 drugs based on the BCS using high throughput solubility assay and permeability assay.

MATERIALS AND METHODS

Materials

Metoprolol, propranolol, dilthiazem, piroxicam, diclofenac, ketoprofen, naproxen, carbamazepine, ketoconazole, danazol, famotidine, atenolol, hydrochlorothiazide, ranitidine, acyclovir, furosemide, L-α-phosphatidylserine (PS), L-α-phosphatidylinositol (PI), and cholesterol (CHO) were obtained from Sigma Chemical (St. Louis, MO). Verapamil was purchased from Wako

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Table 2. BCS class predicted by HTSA and PAMPA.

No.	Compound name	Pred. solubility class[a]	Pred. permeability class[a]	Pred. BCS class[a]	BCS class[b]
1	Metoprolol	H	H	1	1
2	Propranolol	H	H	1	1
3	Dilthiazem	H	H	1	1
4	Piroxicam	H	H	1	2
5	Diclofenac	L	H	2	2
6	Verapamil	H	H	1	2
7	Ketoprofen	L	H	2	2
8	Naproxen	L	H	2	2
9	Carbamazepine	L	H	2	2
10	Ketoconazole	L	L	4	2
11	Danazol	L	H	2	2
12	Famotidine	H	L	3	3
13	Atenolol	H	L	3	3
14	Hydrochlorothiazide	H	L	3	3
15	Ranitidine	H	L	3	3
16	Acyclovir	L	L	4	3
17	Furosemide	L	L	4	4
18	Chlorothiazide	L	L	4	4

[a]Predicted BCS class using HTSA and PAMPA. H and L mean high and low, respectively.
[b]The BCS class was obtained from previously reported values. (From Refs. [13–16].)

Pure Chemical Industries (Osaka, Japan). Chlorothiazide was purchased from Alexis corporation (San Diego, CA). L-α-phosphatidylcholine (PC) and L-α-phosphatidylethanolamine (PE) were obtained from Nippon Oil and Fats Corporation (Tokyo, Japan). 1,7-Octadien was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Other reagents were of analytical grade. The hydrophilic filter plate for solubility assays (Durapore, pore size 0.22 μm) and the hydrophobic filter plate for permeability studies (Durapore, pore size 0.45 μm) were purchased from Millipore corporation (Bedford, MA).

High Throughput Solubility Assay

Simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid without bile acid (SIF, pH 6.8) were prepared according to Japanese Pharmacopoeia (JPXXIV). Two microliters of sample stock solution in DMSO was introduced into each well of 96-well plates, and 200 μL of medium (SGF and SIF) was added. The final DMSO content was approximately 1%. The plate was incubated at 37° C for 20 hours. The solution was filtered using 96-well filter plates. Then, 100 μL of ethanol-water (2:1) was added to 101 μL of the filtrate. Reference solutions were prepared by diluting the sample stock solution to the same composition as the assay solution. The concentration of the assay solution was determined by UV spectroscopy, using the Spectramax 190 (Molecular Devices) at 250–450 nm with intervals of 10 nm. Solubility was calculated from the ratio of the optical absorbance of the assay solution to that of the reference solution.

Parallel Artificial Membrane Permeation Assay

Permeability studies were performed as described previously.[6] In brief, a 96-well microplate (acceptor compartment) was completely filled with pH 6.0, 50 mM sodium phosphate buffer containing 5% DMSO. A hydrophobic filter plate (donor compartment) was fixed on the buffer-filled plate. The filter surface was impregnated with 5 μL of lipid solution, which was composed of PC (0.8%)/PE (0.8%)/PS (0.2%)/PI (0.2%)/CHO (1.0%)/1,7-octadiene (97.0%). A 0.5 mM sample stock solution (100 μL) of the same buffer was added to the filter plate and incubated at 30° C for 1–15 hours. Artificial membrane permeability (P_{am}) was calculated as previously reported.[6] $P_{PAMPA\text{-}PP\text{-}RF}$ was calculated as the paracellular pathway corrected P_{am} based on the Renkin function.[10]

RESULTS AND DISCUSSION

The significance of the solubility to in vivo oral absorption depends on both the permeability and the dose strength.[11] Therefore, the biopharmaceutics classification system (BCS) was introduced to characterize the oral absorption of the solid oral dosage. The

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BCS was originally introduced as a guide to judge the relevancy of the in vitro dissolution test used in the drug development, clinical, and post-marketing stages. However, the BCS was also suggested to be a useful system for characterizing oral drug absorption and to discover drug candidates with appropriate absorption profiles at the early drug discovery stage.

The BCS categorizes drug substances in classes 1 to 4 based on permeability and dose number (Do). Do was calculated from solubility and dose strength using Eq. (1).[11]

$$\text{Do} = \text{Dose strength (weight)}/(\text{Solubility (weight/mL)} \times 250\ \text{mL}) \quad (1)$$

In the following discussion, the dose strength dosed in the United States was used to calculate Do.[12] In the BCS, class 1 drugs have high permeability and high solubility, class 2 drugs have high permeability and low solubility, class 3 drugs have low permeability and high solubility, and class 4 drugs have low permeability and low solubility. According to the Food and Drug Administration (FDA), the boundary between high and low permeability should be 90% absorption in humans, and metoprolol has been suggested as a boundary marker.[13] The boundary between high and low solubility is that the maximum Do over the pH range of 1–7.5 equals 1. However, to strictly classify the drugs according to the BCS, solubility measurements over pH 1–7.5 are required. Moreover, measurements of thermodynamic solubility are required. These requirements are practically impossible at the early drug discovery stage. Therefore, we examined whether HTSA and PAMPA could correctly classify the BCS class.

Simulated gastric fluid and SIF were selected as dissolution media. In the present study, the lower solubility between SGF and SIF was used to calculate Do. The solubility, Do, and permeability of 18 drug compounds measured by HTSA and PAMPA are summarized in Table 1.

As proposed by the FDA, Do = 1 was used as the solubility criterion, and $P_{PAMPA\text{-}PP\text{-}RF}$ of metoprolol was used as the permeability criterion. The previously reported BCS class and predicted class by HTSA and PAMPA are summarized in Table 2.

Fourteen of 18 compounds were correctly classified by SGF solubility, SIF solubility, and PAMPA (78% success rate) (Fig. 1).

The HTSA and PAMPA, which are high throughput assays, could classify the drugs with a high success rate. However, HTSA was performed using only SGF (pH 1.2) and SIF (pH 6.8). This result showed that

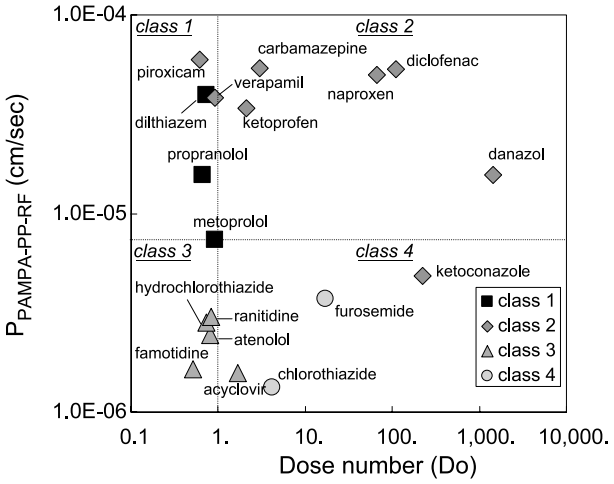

Figure 1. Plot of permeability ($P_{PAMPA\text{-}PP\text{-}RF}$) vs. dose number (Do) obtained by PAMPA and HTSA, respectively. The detection limit values were used when compounds had the detection limit in Table 1.

HTSA could predict BCS class, and PAMPA could also be useful to predict the permeation of drugs.

Piroxicam, verapamil, ketoconazole, and acyclovir were incorrectly classified. Their reported BCS classes are 2, 2, 2, and 3, whereas the predicted classes were 1, 1, 4, and 4, respectively. The Do of piroxicam was slightly underestimated, because it has two pK_as, 1.86 (base), and 5.46 (acid), and, therefore, the pH for the lowest solubility (=highest Do) would be between the two pK_a values.[17] The Do of verapamil was also slightly underestimated, because verapamil has a lower solubility at higher pH than SIF (pH 6.8). The Do in PBS (pH 7.4) was found to be 1 (unpublished data). In the case of ketoconazole, permeability was slightly underestimated, probably because PAMPA was performed in the 30% DMSO condition due to its low solubility.[18] In the case of acyclovir, the exact Do could not be obtained because of its high dose strength and limited solubility to DMSO. Except for these minor cases, HTSA and PAMPA were found to be appropriate methods to classify BCS classes.

CONCLUSION

The result of the present study revealed that in vitro solubility and permeability assays could predict BCS class with a high success rate. The assay methods used in this study (i.e., HTSA and PAMPA) could be performed in a high throughput manner. Therefore, these assays could be useful for rapid evaluation of the oral absorption

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based on the BCS. The in vitro oral absorption information could be delivered to a medicinal chemist at the early stage of drug development, to optimize the pharmacokinetic character. Consequently, this could contribute to an increase in the success rate of preclinical and clinical development.

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